

AMENDMENTS TO THE CLAIMS:

This listing of claims will replace all prior versions, and listings, of claims in the application.

LISTING OF THE CLAIMS:

**1-30. (cancelled)**

**31. (new)** A method of obtaining protein chains from the extracellular hemoglobin molecule of *Arenicola marina*,

the method comprising bringing together a sample of the extracellular hemoglobin molecule of *Arenicola marina* and a dissociation buffer for a time sufficient to separate the protein chains from each other, wherein the dissociation buffer comprises at least one dissociating agent, and optionally, a reducing agent.

**32. (new)** The method according to claim 31, wherein the dissociation buffer comprises about 0.05 M to 0.1 M Trisma (tris(hydroxymethyl)aminomethane) and about 0 to 10 mM EDTA, and has a pH of about 5 to 12.

**33. (new)** The method according to claim 31, wherein the dissociation buffer comprises a reducing agent, and the protein chains are obtained from the reduction of four sub-units of the extracellular hemoglobin molecule.

**34. (new)** A method of preparing primer pairs capable of hybridizing to a nucleic acid encoding the protein chains obtained according to the method as defined in claim 31, the method comprising:

isolating each of the protein chains from the hemoglobin molecule,

microsequencing each of the isolated protein chains to obtain a microsequence corresponding to each of the protein chain sequences, each microsequence comprising 5 to 20 amino acids, and

determining degenerate primer pairs from the microsequences, wherein the degenerate primer pairs are capable of hybridizing to a nucleic acid encoding the protein chains.

**35. (new)** Primer pairs for preparing a nucleic acid encoding the protein chains obtained according to the method as defined in claim 31, the primer pairs selected from the list consisting of:

a) *Sense primer:* GAR TGY GGN CCN TTR CAR CG  
(SEQ ID NO: 21)

*Antisense primer:* CTC CTC TCC TCT CCT CTT CCT  
(SEQ ID NO: 22)

b) *Sense primer:* TGY GGN ATH CTN CAR CG (SEQ ID NO: 23)

*Antisense primer:* CTC CTC TCC TCT CCT CTT CCT  
(SEQ ID NO: 22)

c) *Sense primer:* AAR GTI AAR CAN AAC TGG

(SEQ ID NO: 24)

*Antisense primer:* CTC CTC TCC TCT CCT CTT CCT

(SEQ ID NO: 22)

d) *Sense primer:* TGY TGY AGY ATH GAR GAY CG

(SEQ ID NO: 25)

*Antisense primer:* CTC CTC TCC TCT CCT CTT CCT

(SEQ ID NO: 22)

e) *Sense primer:* AAR GTN ATH TTY GGN AGR GA

(SEQ ID NO: 26)

*Antisense primer:* CTC CTC TCC TCT CCT CTT CCT

(SEQ ID NO: 22)

f) *Sense primer:* GAR CAY CAR TGY GGN GGN GA

(SEQ ID NO: 27)

*Antisense primer:* CTC CTC TCC TCT CCT CTT CCT

(SEQ ID NO: 22)

wherein,

R represents A or G,

Y represents C or T,

N represents A, G, C or T,

I represents inosine, and

H represents A, C or T.

**36. (new)** A method for preparing a nucleotide sequence encoding a protein chain of the extracellular hemoglobin molecule

of *Arenicola marina*, utilizing at least one of the primer pairs of claim 35, the method comprising a polymerase chain reaction (PCR) of at least 30 cycles of the following steps:

denaturing cDNA encoding one of the protein chains so as to denature any secondary structures and RNA residuals, the cDNA being obtained from mRNA, to obtain strands of denatured monocatenary cDNA,

hybridizing the primer pair to the strands of denatured monocatenary cDNA at an appropriate temperature, to obtain hybridized primers, and

synthesizing a complementary strand of the cDNA using a polymerase at an appropriate temperature.

**37. (new)** A protein encoded by a nucleotide sequence obtained according to the method as defined in claim 36.

**38. (new)** The protein according to claim 37, wherein the protein comprises:

the sequence SEQ ID NO: 2,

or any sequence derived from the sequence SEQ ID NO: 2 by substitution, suppression or addition of one or more amino acids of SEQ ID NO: 2, provided that the derived sequence allows the transport of oxygen,

or any sequence homologous to the sequence SEQ ID NO: 2 having a homology of at least 75% with the sequence SEQ ID NO: 2,

provided that the homologous sequence allows the transport of oxygen,

or any fragment of one of the sequences defined above, provided that the fragment allows the transport of oxygen.

**39. (new)** The protein according to claim 37, wherein the protein comprises:

the sequence SEQ ID NO: 4,

or any sequence derived from the sequence SEQ ID NO: 4 by substitution, suppression or addition of one or more amino acids of SEQ ID NO: 4, provided that the derived sequence allows the transport of oxygen,

or any sequence homologous to the sequence SEQ ID NO: 4 having a homology of at least 75% with the sequence SEQ ID NO: 4, provided that the homologous sequence allows the transport of oxygen,

or any fragment of one of the sequences defined above, provided that the fragment allows the transport of oxygen.

**40. (new)** The protein according to claim 37, wherein the protein comprises:

the sequence SEQ ID NO: 6,

or any sequence derived from the sequence SEQ ID NO: 6 by substitution, suppression or addition of one or more amino

acids of SEQ ID NO: 6, provided that the derived sequence allows the transport of oxygen,

or any sequence homologous to the sequence SEQ ID NO: 6 having a homology of at least 75% with the sequence SEQ ID NO: 6, provided that the homologous sequence allows the transport of oxygen,

or any fragment of one of the sequences defined above, provided that the fragment allows the transport of oxygen.

**41. (new)** The protein according to claim 37, wherein the protein comprises:

the sequence SEQ ID NO: 8,

or any sequence derived from the sequence SEQ ID NO: 8 by substitution, suppression or addition of one or more amino acids of SEQ ID NO: 8, provided that the derived sequence allows the transport of oxygen,

or any sequence homologous to the sequence SEQ ID NO: 8 having a homology of at least 75% with the sequence SEQ ID NO: 8, provided that the homologous sequence allows the transport of oxygen,

or any fragment of one of the sequences defined above, provided that the fragment allows the transport of oxygen.

**42. (new)** The protein according to claim 37, wherein the protein comprises:

the sequence SEQ ID NO: 10,

or any sequence derived from the sequence SEQ ID NO: 10 by substitution, suppression or addition of one or more amino acids of SEQ ID NO: 10, provided that the derived sequence allows the transport of oxygen,

or any sequence homologous to the sequence SEQ ID NO: 10 having a homology of at least 75% with the sequence SEQ ID NO: 10, provided that the homologous sequence allows the transport of oxygen,

or any fragment of one of the sequences defined above, provided that the fragment allows the transport of oxygen.

**43. (new)** The protein according to claim 37, wherein the protein comprises:

the sequence SEQ ID NO: 12,

or any sequence derived from the sequence SEQ ID NO: 12 by substitution, suppression or addition of one or more amino acids of SEQ ID NO: 12, provided that the derived sequence allows the transport of oxygen,

or any sequence homologous to the sequence SEQ ID NO: 12 having a homology of at least 75% with the sequence SEQ ID NO: 12, provided that the homologous sequence allows the transport of oxygen,

or any fragment of one of the sequences defined above, provided that the fragment allows the transport of oxygen.

**44. (new)** A nucleotide sequence obtained according to the method as defined in claim 36.

**45. (new)** A nucleotide sequence encoding a protein as defined in claim 37.

**46. (new)** The nucleotide sequence according to claim 45, comprising

the nucleotide sequence SEQ ID NO: 1 encoding a protein represented by SEQ ID NO: 2,

or any nucleotide sequence derived by degeneration of the genetic code from the sequence SEQ ID NO: 1 and encoding a protein represented by SEQ ID NO: 2,

or any nucleotide sequence derived by substitution, suppression or addition of one or more nucleotides from the sequence SEQ ID NO: 1 encoding a protein derived from SEQ ID NO: 2,

or any nucleotide sequence homologous to SEQ ID NO: 1 having a homology of at least 60% with the sequence SEQ ID NO: 1,

or any fragment of the nucleotide sequence SEQ ID NO: 1 or of the nucleotide sequences defined above,

or any nucleotide sequence complementary to one of the above sequences or fragments,



or any nucleotide sequence capable of hybridizing under stringent conditions with the nucleotide sequence complementary to one of the above sequences or fragments.

**47. (new)** The nucleotide sequence according to claim 45, comprising

the nucleotide sequence SEQ ID NO: 3 encoding a protein represented by SEQ ID NO: 4,

or any nucleotide sequence derived by degeneration of the genetic code from the sequence SEQ ID NO: 3 and encoding a protein represented by SEQ ID NO: 4,

or any nucleotide sequence derived by substitution, suppression or addition of one or more nucleotides from the sequence SEQ ID NO: 3 encoding a protein derived from SEQ ID NO: 4,

or any nucleotide sequence homologous to SEQ ID NO: 3 having a homology of at least 60% with the sequence SEQ ID NO: 3,

or any fragment of the nucleotide sequence SEQ ID NO: 3 or of the nucleotide sequences defined above,

or any nucleotide sequence complementary to one of the above sequences or fragments,

or any nucleotide sequence capable of hybridizing under stringent conditions with the nucleotide sequence complementary to one of the above sequences or fragments.

**48. (new)** The nucleotide sequence according to claim 45, comprising

the nucleotide sequence SEQ ID NO: 5 encoding a protein represented by SEQ ID NO: 6,

or any nucleotide sequence derived by degeneration of the genetic code from the sequence SEQ ID NO: 5 and encoding a protein represented by SEQ ID NO: 6,

or any nucleotide sequence derived by substitution, suppression or addition of one or more nucleotides from the sequence SEQ ID NO: 5 encoding a protein derived from SEQ ID NO: 6,

or any nucleotide sequence homologous to SEQ ID NO: 5 having a homology of at least 60% with the sequence SEQ ID NO: 5,

or any fragment of the nucleotide sequence SEQ ID NO: 5 or of the nucleotide sequences defined above,

or any nucleotide sequence complementary to one of the above sequences or fragments,

or any nucleotide sequence capable of hybridizing under stringent conditions with the nucleotide sequence complementary to one of the above sequences or fragments.

**49. (new)** The nucleotide sequence according to claim 45, comprising

the nucleotide sequence SEQ ID NO: 7 encoding a protein represented by SEQ ID NO: 8,

or any nucleotide sequence derived by degeneration of the genetic code from the sequence SEQ ID NO: 7 and encoding a protein represented by SEQ ID NO: 8,

or any nucleotide sequence derived by substitution, suppression or addition of one or more nucleotides from the sequence SEQ ID NO: 7 encoding a protein derived from SEQ ID NO: 8,

or any nucleotide sequence homologous to SEQ ID NO: 7 having a homology of at least 60% with the sequence SEQ ID NO: 7,

or any fragment of the nucleotide sequence SEQ ID NO: 7 or of the nucleotide sequences defined above,

or any nucleotide sequence complementary to one of the above sequences or fragments,

or any nucleotide sequence capable of hybridizing under stringent conditions with the nucleotide sequence complementary to one of the above sequences or fragments.

**50. (new)** The nucleotide sequence according to claim 45, comprising

the nucleotide sequence SEQ ID NO: 9 encoding a protein represented by SEQ ID NO: 10,

or any nucleotide sequence derived by degeneration of the genetic code from the sequence SEQ ID NO: 9 and encoding a protein represented by SEQ ID NO: 10,

or any nucleotide sequence derived by substitution, suppression or addition of one or more nucleotides from the sequence SEQ ID NO: 9 encoding a protein derived from SEQ ID NO: 10,

or any nucleotide sequence homologous to SEQ ID NO: 9 having a homology of at least 60% with the sequence SEQ ID NO: 9,

or any fragment of the nucleotide sequence SEQ ID NO: 9 or of the nucleotide sequences defined above,

or any nucleotide sequence complementary to one of the above sequences or fragments,

or any nucleotide sequence capable of hybridizing under stringent conditions with the nucleotide sequence complementary to one of the above sequences or fragments.

**51. (new)** The nucleotide sequence according to claim 45, comprising

the nucleotide sequence SEQ ID NO: 11 encoding a protein represented by SEQ ID NO: 12,

or any nucleotide sequence derived by degeneration of the genetic code from the sequence SEQ ID NO: 11 and encoding a protein represented by SEQ ID NO: 12,

or any nucleotide sequence derived by substitution, suppression or addition of one or more nucleotides from the sequence SEQ ID NO: 11 encoding a protein derived from SEQ ID NO: 12,

or any nucleotide sequence homologous to SEQ ID NO: 11 having a homology of at least 60% with the sequence SEQ ID NO: 11,

or any fragment of the nucleotide sequence SEQ ID NO: 11 or of the nucleotide sequences defined above,

or any nucleotide sequence complementary to one of the above sequences or fragments,

or any nucleotide sequence capable of hybridizing under stringent conditions with the nucleotide sequence complementary to one of the above sequences or fragments.

**52. (new)** A method for preparing nucleotide sequences encoding a protein chain of extracellular hemoglobin molecule of *Arenicola marina*, the method comprising the following steps:

bringing together a sample of the hemoglobin molecule and a dissociation buffer for a time sufficient to separate the protein chains from each other, wherein the dissociation buffer comprises at least one dissociating agent and a reducing agent,

dissociating and reducing the hemoglobin molecule to obtain the protein chains,

isolating each of the protein chains,

microsequencing each of the isolated protein chains to obtain a microsequence corresponding to each of the protein chain sequences, each microsequence comprising 5 to 20 amino acids,

determining degenerate primer pairs from the microsequence, wherein the degenerate primer pairs are capable of hybridizing to nucleic acid encoding the protein chains,

preparing nucleotide sequences encoding the protein chains, utilizing the degenerate primer pairs, by a polymerase chain reaction (PCR) comprising an initial step of denaturing for approximately 10 seconds to approximately 5 minutes at a temperature between approximately 90°C and approximately 110°C, followed by 30 to 40 cycles of the following steps:

denaturing for approximately 10 seconds to approximately 5 minutes at a temperature between approximately 90°C and approximately 110°C,

hybridizing for approximately 20 seconds to approximately 2 minutes at a temperature between approximately 50°C and approximately 56°C,

elongating for approximately 20 seconds to approximately 90 seconds at a temperature between approximately 70°C and approximately 75°C, and

a further final step comprising elongation of approximately 5 minutes to approximately 15 minutes at a temperature between approximately 70°C and approximately 75°C.

**53. (new)** An isolated protein comprising an amino acid sequence of SEQ ID NO: 2,

or any sequence derived from the sequence SEQ ID NO: 2 by substitution, suppression or addition of one or more amino acids of SEQ ID NO: 2, provided that the derived sequence allows the transport of oxygen,

or any sequence homologous to the sequence SEQ ID NO: 2 having a homology of at least 75% with the sequence SEQ ID NO: 2, provided that the homologous sequence allows the transport of oxygen,

or any fragment of one of the sequences defined above, provided that the fragment allows the transport of oxygen.

**54. (new)** An isolated protein encoded by a nucleic acid comprising a nucleotide sequence of SEQ ID NO: 1,

or any nucleotide sequence derived by degeneration of the genetic code from the sequence SEQ ID NO: 1,

or any nucleotide sequence derived by substitution, suppression or addition of one or more nucleotides from the sequence SEQ ID NO: 1, provided that the protein allows the transport of oxygen,

or any nucleotide sequence homologous to SEQ ID NO: 1 having a homology of at least 60% with the sequence SEQ ID NO: 1, provided that the protein allows the transport of oxygen,

or any fragment of the nucleotide sequence SEQ ID NO: 1 or of the nucleotide sequences defined above, provided that the protein allows the transport of oxygen.

**55. (new)** The method according to claim 31, wherein the dissociation buffer comprises dithiothreitol (DTT), tris(2-carboxyethyl)phosphine hydrochloride (TCEP), beta-mercaptoethanol, or any combination thereof.

**56. (new)** The method according to claim 32, wherein the dissociation buffer has a pH of about 7.5 to 12.

**57. (new)** The protein according to claim 53, wherein the fragment comprises at least 60 amino acids.

**58. (new)** The protein according to claim 53, wherein the fragment comprises at least 160 contiguous amino acids of SEQ ID NO: 2.

**59. (new)** The method according to claim 52, wherein the dissociation buffer comprises dithiothreitol (DTT), tris(2-carboxyethyl)phosphine hydrochloride (TCEP), beta-mercaptoethanol, or any combination thereof.